HELLER EHRMAN WHITE & MCAULIFFE LLP
Sheet 1 of 23
Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
        AND USES THEREOF FOR NESTED SORTING AND
        HIGH THROUGHPUT SCREENING.
Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

Sorting by pools

FIG. I

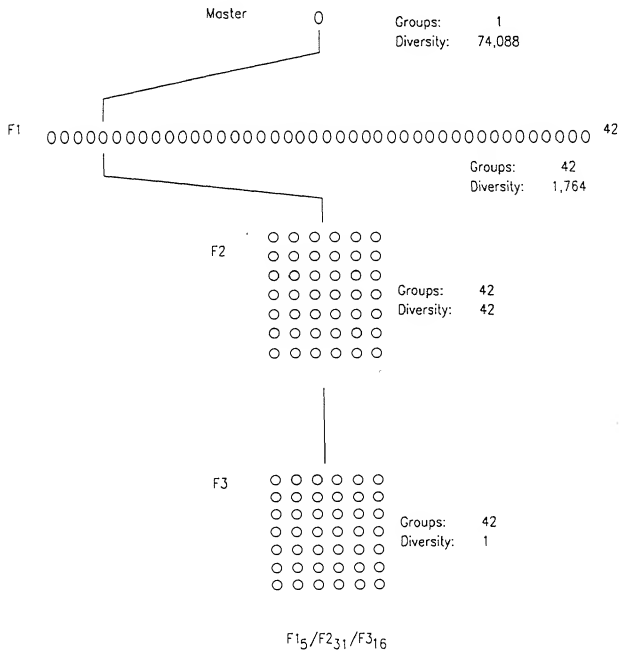## Sorting by pools: Decreasing pool diversities

| Master | 0 | Groups: | 1 |
| | | Diversity: | 74,088 |

F1 OOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO 42

Groups: 42
Diversity: 1,764

F2

Groups: 42
Diversity: 42

F3

Groups: 42
Diversity: 1

$F1_5/F2_{31}/F3_{16}$

FIG. 2

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Sorting by pools: Screening large divrsity libraries

Libraries		Pools		
F1	100	1,000	100	1,000
F2	100	100	1,000	1,000
F3	100	100	1,000	1,000
Totals	10^6	10^7	10^8	10^9
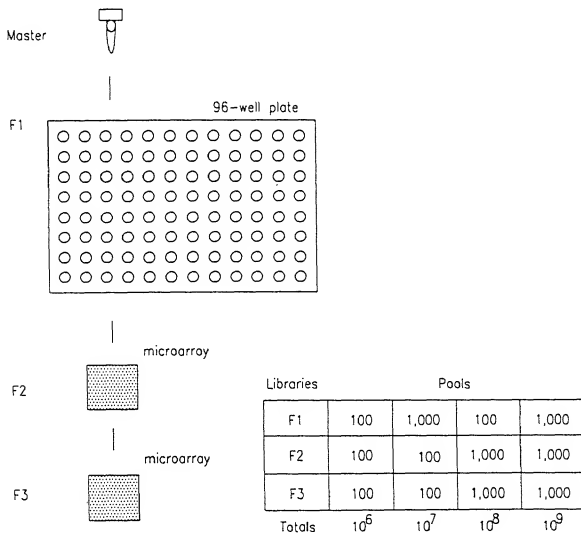
FIG. 3

Searching a mutation library
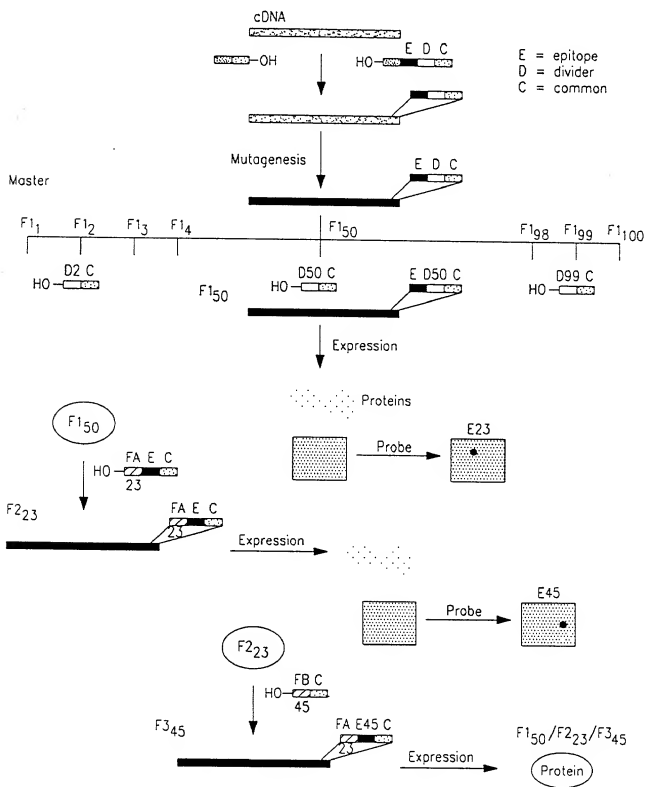
FIG. 4

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Making a recombinant antibody library

FIG. 5

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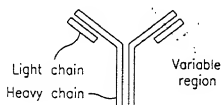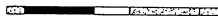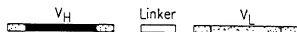
Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs
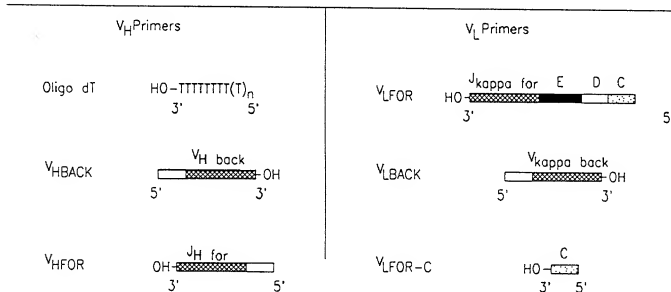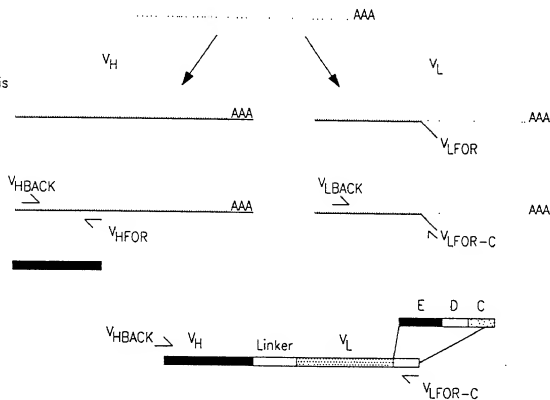
2. cDNA synthesis

3. amplification

4. assembly

FIG. 6

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Serial No 09/910,120 Filed: July 18, 2001
Our Docket No : 25885-17-1

Creating the master antibody library: Linker addition

1. mRNA purification from spleen or PBLs
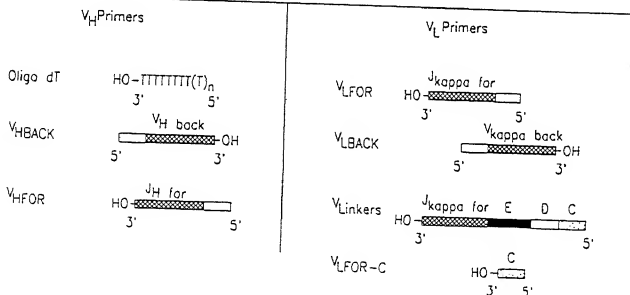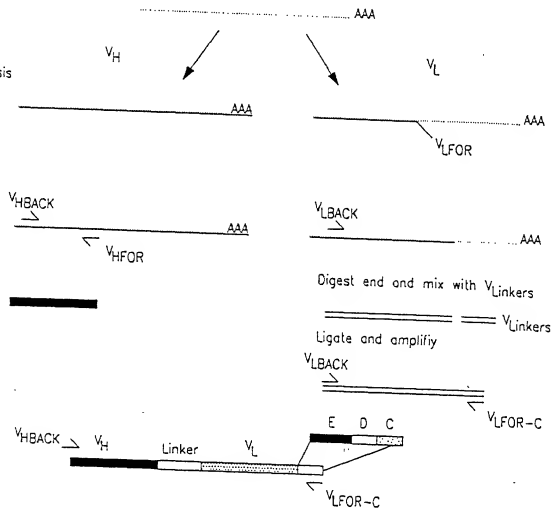
2. cDNA synthesis

3. amplification

4. assembly

V_H Primers

Oligo dT

V_{HBACK}

V_{HFOR}

V_L Primers

V_{LFOR}

V_{LBACK}

$V_{Linkers}$

V_{LFOR-C}

FIG. 7

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Searching a recombinant antibody library

FIG. 8

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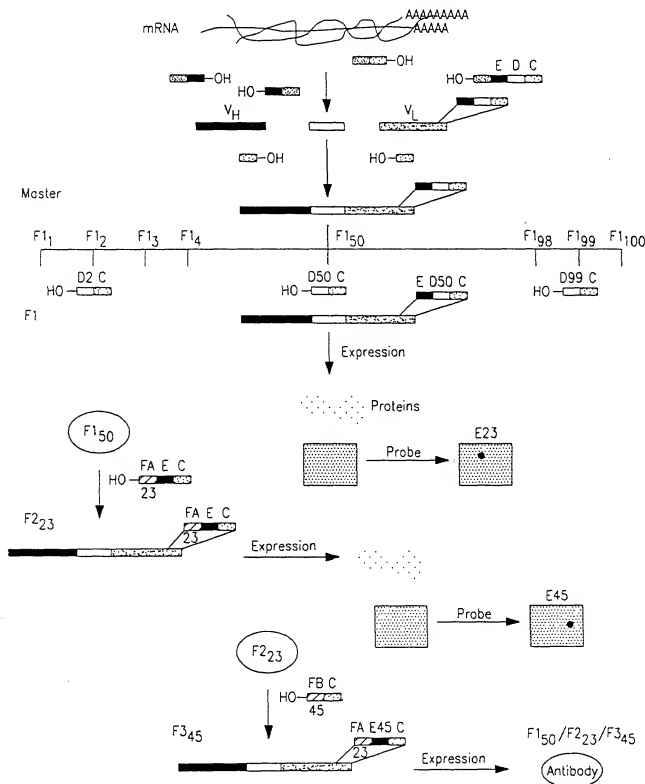
Physical elements to include in the kits and combinations

- *Anti-tag Arrays*™

- Primer sets

$D_1 - D_{1,000}$

$D_1 - D_{1,000}$

$FA_1 - FA_{1,000}$

$FB_1 - FB_{1,000}$

- Readers

- Software

FIG. 9

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Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
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Applicant: Ault-Riché et al.
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

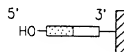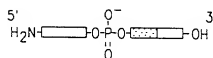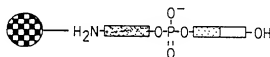## Making the V_{LFOR} primers: Solid phase synthesis
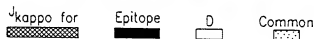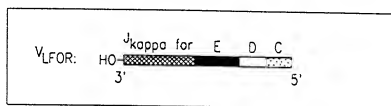
1. Synthesize oligo on solid support

2. Add aminolink prior to cleavage

3. Couple to tosyl activated magnetic beads
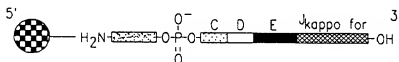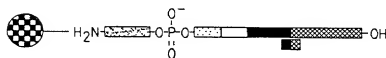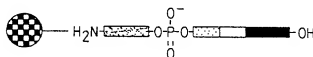
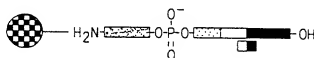4. Extended by hybridizing with DNA patch and ligating

FIG. 10

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AND USES THEREOF FOR NESTED SORTING AND
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Making the V_{LFOR} primers: Overlapping hybridiation
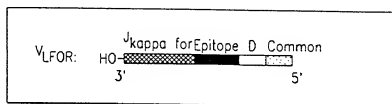
- Synthesize 4,028 different oligos:
  (26 for $J_{kappa\ for}$; 2,000 for Epitope, 2,000 for D; 2 for Common

2. Assemble oligos for + and − strands of the different regions
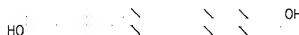
3. Ligose the assembled oligos

4. 1st strand synthesis with biotinyloted primer
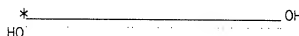
- 2nd strand synthesis with non−biotinyloted primer

6. Bind to ovidin coated mognetic beads and then denature

7. Purify non−biotinyloted ssDNA

FIG. 11
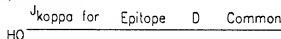
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Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
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Applicant: Ault-Riché et al.
Serial No. 09/910,120 Filed: July 18, 2001
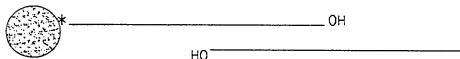Our Docket No.: 25885-1751

Building the collection of antibody/tag pairs: Hybridoma screening

Stable hybridoma cells

Isolated monoclonals
grown in 96-well plates
(quantify Abs in culture
supernatants by ELISA)

Purified antibodies
(purify with protein L plates;
quantify purified antibodies by ELISA)

Individual protein
preparations

Array onto filter
(up to 10,000 per filter)

Pooled protein
preparations

Bind purified antibodies to
magnetic beads coated with
anti-mouse Ig mAb (Dynal)

Pan a random disulfide-constrained
phage display library against the beads
(4 rounds with plate amplifications)

Bind enriched phage library to filter;
Stain with anti-phage mAb-HRP;
Image with CCD-based system

Cut out best spots
Recover and propagate phage
(or PCR amplify DNA)

Sequence DNA to
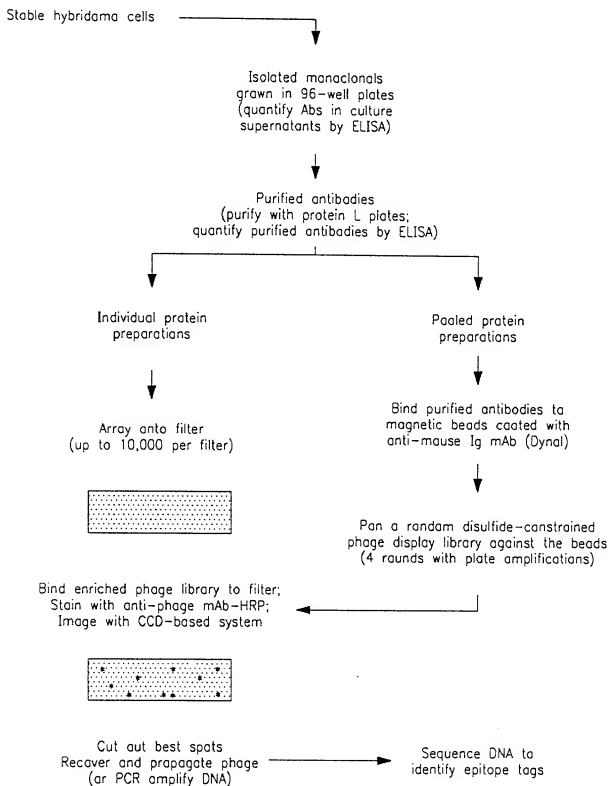identify epitope tags

FIG. 12

Table 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR
    A. Human VH back primers (sense)

        HuVH1aBACK   5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
        HuVH2aBACK   5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
        HuVH3aBACK   5'-GAG GTG CAG CTG GTG CAG TCT GG-3'
        HuVH4aBACK   5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
        HuVH5aBACK   5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
        HuVH6aBACK   5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

    B. Human JH forward primers (anti-sense)

        HuJH1-2FOR   5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
        HuJH3FOR   5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
        HuJH4-5FOR   5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
        HuJH6FOR   5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

    C. Human V kappa back primers (sense)

        HuVk1aBACK   5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
        HuVk2aBACK   5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
        HuVk3aBACK   5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
        HuVk4aBACK   5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
        HuVk5aBACK   5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
        HuVk6aBACK   5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

    C. Human V lambda back primers (sense)

        HuVλ1BACK   5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
        HuVλ2BACK   5'-CAG TCT GCC GTG ACT CAG CCT GG-3'
        HuVλ3aBACK   5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
        HuVλ3bBACK   5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
        HuVλ4BACK   5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
        HuVλ5BACK   5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
        HuVλ6BACK   5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

    D. Human J kappa forward primers (anti-sense)

        HuJk1FOR   5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
        HuJk2FOR   5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
        HuJk3FOR   5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
        HuJk4FOR   5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
        HuJk5FOR   5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

    D. Human J. lambda forward primers (anti-sense)

        HuJλ1FOR   5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
        HuJλ2-3FOR   5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
        HuJλ4-5FOR   5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

FIG. 13A

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Applicant: Ault-Riche *et al.*
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Our Docket No : 25885-1751

2. Linker fragment PCR

   F. Reverse JH for scFv linker (sense)

```
                    ————FR4 heavy ——————————|—linker
    RHuJH1-2    5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
    RHuJH3      5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
    RHuJH4-5    5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
    RHuJH6      5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'
```

   F. Reverse Vk for scFv linker (anti-sense)

```
                       ————FR1 light ————————|—linker
    RHuVk1aBACKFv   5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC CC-3'
    RHuVk2aBACKFv   5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
    RHuVk3aBACKFv   5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
    RHuVk4aBACKFv   5'-GG AGA CTG GGT CAT CAC CAT CGA TCC GCC-3'
    RHuVk5aBACKFv   5'-GG AGA CTG CGT GAG TGT GGT TTC CGA TCC GCC-3'
    RHuVk6aBACKFv   5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'
```

   F. Reverse Vλ for scFv linker (anti-sense)

```
                        ————FR1 light ——————————|————linker
    RHuVλBACK1Fv    5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
    RHuVλBACK2Fv    5'-GC AGG CTG AGT CAG AGC AGA CTC CGA TCC GCC ACC GCC AGA G-3'
    RHuVλBACK3aFv   5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
    RHuVλBACK3bFv   5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
    RHuVλBACK4Fv    5'-GG CGG TTG AGT CAG TAT AAC GTC CGA TCC GCC ACC GCC AGA G-3'
    RHuVλBACK5Fv    5'-GA CGG CTG AGT CAG CAG CAG CTC CGA TCC GCC ACC GCC AGA G-3'
    RHuVλBACK6Fv    5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'
```

3. Pull-through primers for introduction of restriction sites*

   G. Human VH back (Sfi)primers (sense)

```
    HuVH1aBACKSfi                                           |———FR1 heavy ——————
    5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
    HuVH2aBACKSfi
    5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'
    HuVH3aBACKSfi
    5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG CAG TCT GG-3'
    HuVH4aBACKSfi
    5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
    HuVH5uBACKSfi
    5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GG-3'
    HuVH6aBACKSfi
    5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'
```

   H. Human J kappa forward (Not) primers (anti-sense)

```
    HuJk1FORNot                    |———FR4 light ——————————
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3'
    HuJk2FORNot
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAG CTT GGT CCC-3'
```

   H. Human J kappa forward (Not) primers (anti-sense)(continued)

```
    HuJk3FORNot                    |———FR4 light ——————————
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3'
    HuJk4FORNot
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
    HuJk5FORNot
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3'
```

   H. Human J lambda forward (Not) primers (anti-sense)
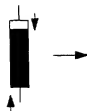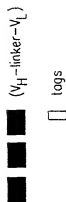
```
    HuJl1FORNOT                                 FR4 light
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
    HuJl2-3FORNOT
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
    HuJl4-5FORNOT
    5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAA AAC GGT GAC CTG GGT CCC-3'
```

*Recognition site for restriction enzyme is underlined.   FIG. 13B

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step I

Tag and assemble immunoglobulin genes

$(V_H-linker-V_L)$

tags

Create 1,000 sub-libraries by separate PCR amplification
reactions using tag-specific PCR primers
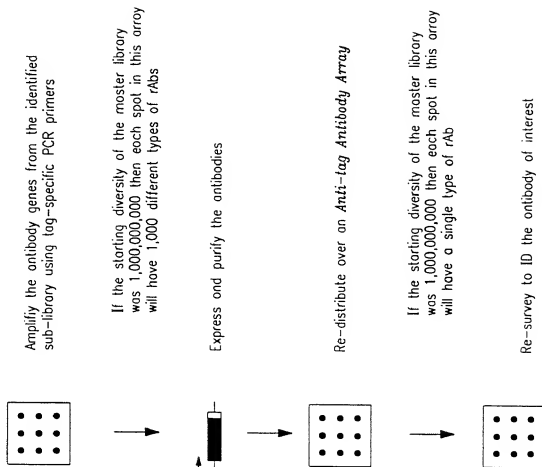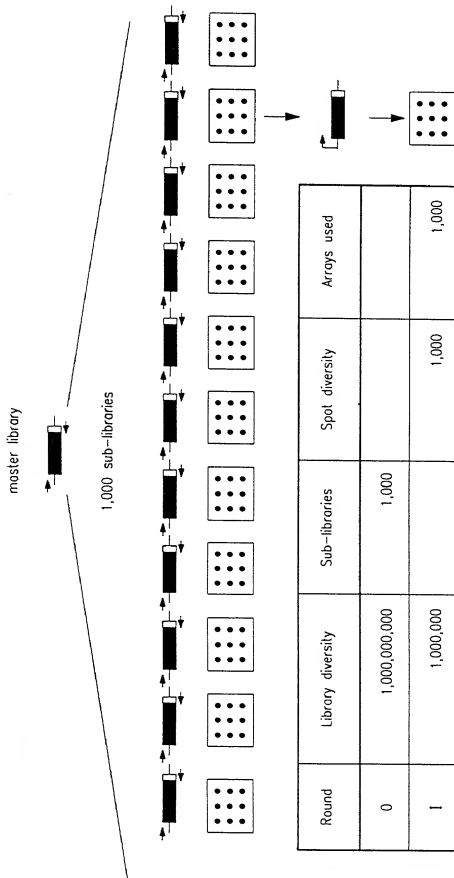
1,000 sub-libraries

FIG. 14A

step II

express antibodies from a sub-library and
bathe over on *Anti-tag Antibody Array*

bathe array with tagged antigen

Anti-tag
antibodies

Anti-tag antibody
with captured and
tagged antibodies
from a sub-library

ID spot containing the antigen
with a labeled developing Ab

FIG. 14B

step III

Amplify the antibody genes from the identified
sub-library using tag-specific PCR primers

If the starting diversity of the master library
was 1,000,000,000 then each spot in this array
will have 1,000 different types of rAbs

Express and purify the antibodies

Re-distribute over an *Anti-tag Antibody Array*

If the starting diversity of the master library
was 1,000,000,000 then each spot in this array
will have a single type of rAb

Re-survey to ID the antibody of interest

FIG. 14C

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FIG. 14D

Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIG. 15
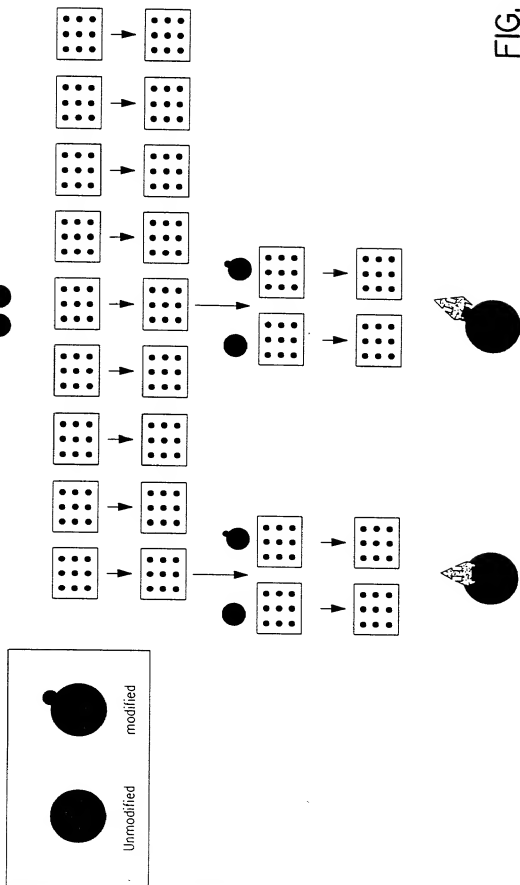
FIG. 16

Simultaneous searches

3 Arrays per Ag

Round	Arrays	Bait Probe
I	1,000 Abs Ags	
II	1,000 Abs Ags	
III	1,000 Ags Abs / 3,000	
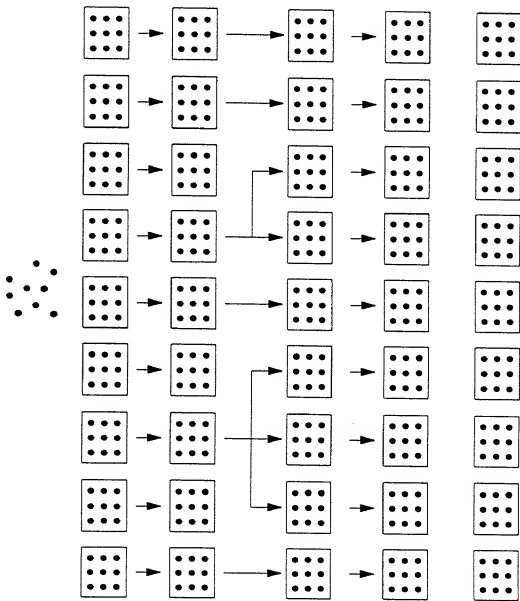
FIG. 17

Protein interaction mapping

cDNA library

• Human tissue

• pathogen

• yeast

Generate a tagged cDNA library

Create sub-libraries by PCR

Distribute onto arrays

Probe arrays with one or several labeled proteins, peptides, or drugs

FIG. 18

FIG. 19